Engineering Anaerobic Gut Fungi for Lignocellulose Breakdown

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Project Goals: The goal of this project is to engineer anaerobic gut fungi as novel platform organisms for biofuel production from plant material. To accomplish this goal, a panel of anaerobic fungi will be isolated from different herbivores and screened for their ability to degrade lignocellulose. The basic metabolic networks that govern lignocellulose hydrolysis within anaerobic fungi will also be determined, and models will be generated to describe how important enzyme groups are coordinated during breakdown. Using this information, genetic transformation strategies to manipulate gut fungi will be developed, which would endow them with enhanced functionality against a range of industrially relevant substrates. Collectively, this information will establish the molecular framework for anaerobic fungal hydrolysis, and will guide in the development of lignocellulosic biofuels.

To support renewable technologies, it is necessary to develop more efficient methods to extract sugars from crude plant biomass (lignocellulose). While plants contain cellulose that depolymerizes into fermentable sugars for microbial biofuel production, it is trapped within lignin, hemicellulose and other biopolymers that complicate its hydrolysis. To address this issue, one can turn to nature, particularly to microbes that routinely degrade plant biomass. Many large herbivores, such as cows and horses, harbor a consortium of microbes in their digestive tracts that convert recalcitrant biomass into sugars. Within this consortium, anaerobic gut fungi are the primary colonizers of plant material, and represent a rich source of biomass degrading enzymes. We have used transcriptomics to identify biomass degrading enzymes produced by the gut fungi and, moreover, have examined regulation patterns to determine how the suite of enzymes is tailored to the carbon source present.

By providing a pulse of a simple sugar during growth on un-pretreated biomass we have studied how the transcriptome remodels to match the perturbation. This reveals how quickly gut fungi

respond to alter the expression of biomass degrading enzymes when they are no longer necessary. Additionally, patterns of co-regulated transcripts may provide insight into the putative function of transcripts with unknown function. Cluster analysis of transcriptomic regulation data reveals co-regulated groups enriched in biomass degrading function, primarily consisting of cellulolytic and/or hemicellulolytic enzymes. Further work examining the transcriptome on a variety of substrates ranging in complexity, including glucose, cellobiose, cellulose, and biomass provides further insight to how these genes are regulated. Gene set enrichment analysis reveals not only how the expression of biomass degrading genes changes across these substrates, but also how the variety of biomass degrading enzymes changes. In the case of two isolated strains, *Neocallimastix californiae* and *Piromyces finnis*, there is a gradual increase in variety of biomass degrading enzymes with the increase in complexity of the carbon source. However, in the case of a third isolated strain, *Anaeromyces robustus*, GSEA reveals that cellobiose triggers the increase in expression of a wide variety of enzymes, not isolated to those involved in hydrolyzing cellobiose and cellulose. Further, we can examine the changes in expression of core metabolic proteins to determine how the fungal cells tailor their metabolism across these different conditions.

Recently, in partnership with the JGI, we have acquired genomic information for each of these isolates. Combining the regulation information with genomic localization, we can begin to identify potential promoters that control expression of these important genes. By investigating the DNA regions upstream of genes of similar functions and similar regulation patterns, we are currently working to identify consensus sequences that may be controlled by the same transcription factors. Together, this approach will enhance our efforts to develop new tools to genetically engineer the anaerobic fungi.

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